EFFECT OF DIETARY VITAMIN E AND SELENIUM ON GROWTH PERFORMANCE AND IMMUNE RESPONSE OF NURSERY PIGS FOLLOWING AN IMMUNE CHALLENGE

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Abstract: An experiment was conducted to evaluate the effects of selenium and vitamin E on growth performance and immune response of nursery pigs. To study the acute immune response, pigs were subjected to an acute immune challenge by a single intraperitoneal injection of lipopolysaccharide (LPS). Two hundred eighty mixed sex piglets (PIC 380) breed with an initial BW of 5.8 kg were used in a 36 d experiment. Pigs were housed 10 pigs/pen (5 gilts and 5 barrows), piglets were blocked and stratified based on sex and BW. Pigs and feeders were weighed once per WK to determine ADG, ADFI and G:F ratio. Serum samples were used to analyze tumor necrosis factor TNF-α and interleukin IL-1 concentrations using ELISA Kit, and plasma samples were used to determine the enzyme Glutathione peroxidase activity.

On d 21 of the study, 4 pigs from each pen were challenged with LPS *E.coli* O111:B4 suspended in a 9 g/L of sterile saline solution for a final dosage of 25 µg of LPS/kg of BW. Between d 8 and 21, the results indicate that vitamin E and selenium did not improve ADG, ADFI, and G:F . Between d 21 and 36, pigs supplemented with selenium and both Se. 0.3 and vitamin E 32 IU improved ADG and G:F efficiency. Overall, G:F was improved (P=0.04) in pigs fed 0.3 mg/kg of selenium and vitamin 32 IU compared to pigs fed 0.15 mg/kg and vitamin E 16 IU/kg. This improvement might be attributed to selenium utilization during protein turnover for the synthesis of sulfur-containing amino acids. Following LPS challenge, there was an increase production of proinflammatory cytokines such as TNF-α and IL-1β. A numeric decrease of TNF-α and IL-1β was observed in pigs fed additional Se and vitamin E. However, no statistical difference was observed among the dietary treatments.The benefits of decreasing production of cytokines in pigs fed additional Se and vitamin E following LPS challenge strengthens the concept that antioxidants can enhance and prevent depressions in performance under periods of stress. More study in this area is needed to better understand the required amount of vitamin E to improve the immune response of nursery pigs and growth performance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Piglets undergo significant environmental, physiological, and immunological challenges during the weaning period. Thus, exposing weaned pigs to diseases, this can negatively affects performance. The weaning period is the most stressful period in the life of a pig. The piglets must cope with these stressors to allow normal growth. One of the challenges weaned pigs have to overcome during the weaning period is the change in their diet, from liquid milk to solid feed, and change in environment, from farrowing barn to the nursery building. (Campbell et al., 2013). Piglets are weaned when their maternal immunity is still high. At birth, piglets are healthy, and maternal milk intake provides immunity to the piglets. The type of post-weaning diet has an influence on intestinal morphology (Leiting et al., 1998). Makkink et al (1994) reported that the weaning period is followed by physiological and architectural changes such as development of the intestines. Because of the change in form of feed and physiological changes in structure and function of intestinal enzymes at weaning, feed intake usually decreases.

To overcome these stressors occurring during the weaning period, scientists over the past decade have been investigating the health benefits of antioxidant substances such as vitamin E and selenium in animal nutrition. More investigation about selenium and vitamin E, two important members of the antioxidants family is needed to better

understand their interaction. Swine research has played an important role in this area, particularly to improve animal health and production efficiency (Oldfield, 1998). Several studies have reported the beneficial effects of supplementation of vitamin E and selenium in livestock species. Moreover, research around the globe has reported that animal species are vulnerable to selenium toxicity and deficiency. The deficiency symptoms can include white muscle disease and mulberry heart disease in swine (Blood et al., 1983). Mahan et al. (2000) reported that blood and tissue vitamin E concentrations of pigs decline dramatically during the weaning period. At this phase, piglets are most susceptible to vitamin E deficiency problems. About two percent of young pigs die during this post-weaning period because of low levels of vitamin E and or selenium received from the sow. Selenium serves a similar purpose as vitamin E in pigs. Despite the high level of Vitamin E and selenium within the allowable level (0.3 mg/kg of diet) used in formulating swine diet, deficiency of both vitamin E and Selenium is still occurring on many swine farms. Deficiency symptoms mostly occur during reproduction and the nursery phase. The benefit of these nutrients on disease resistance is demonstrated by enhancing the immune function and protecting the cell against peroxidation by the action of the enzyme Glutathione peroxidase, which destroys free radicals. Deficiencies of these nutrients can compromise the immune system; thus, decreasing performance and production (Finch et al., 1996).

Vitamin E is a fat-soluble vitamin that has several functions. α-tocopherol is an active form of vitamin E and serves as a component of cell membranes (Morrisey, 1994). The intracellular transport of vitamin E in the liver appears to involve specific tocopherol-binding proteins. Vitamin E is an important constituent of all the membranes

found in cells, including the plasma, mitochondrial and nuclear membrane. Vitamin E is the major chain-breaking antioxidant in body tissues and is considered the first line of defense against lipid peroxidation, protecting cell membranes at an early stage of free radical attack through its free radical scavenging activity. If vitamin E is absent from the diet, one would predict damage to the cell membranes of immune and reproductive cells (Borneboe et al., 1990).

Selenium is an essential trace mineral routinely added in all swine diets via trace mineral premixes. Pigs require selenium in diet and used as a component of an enzyme (glutathione peroxidase) that protects membranes at the cellular and subcellular level against lipid peroxide damage. Selenium also has been shown to be involved in the metabolism of thyroid hormones, which play a central role in the regulation and coordination of metabolism (Mahan et al., 1980).

The current selenium requirements for pigs, as established by the National Research Committee on Swine Nutrition (2012), range from 0.3 mg/kg in nursery pigs to 0.15 mg/kg in finishing pigs and breeding sows. Selenium is very toxic, slightly higher (0.6 mg/kg) concentrations can be toxic. The Feed and Drug Administration (FDA) regulates dietary additions of selenium in swine diets in the U.S. The current FDA regulations allow up to 0.3 mg/kg of added selenium in the diets for all classes of swine (Mavromichalis, 2014).

Several studies have reported a close relationship between vitamin E and Selenium within tissues. Selenium has a sparing effect on vitamin E, preserves the pancreas integrity for normal fat digestion and reduces the amount of vitamin E needed to

maintain lipid membranes, thereby delaying the onset of deficiency symptoms. Likewise, vitamin E and sulfur-containing amino acids partially protect against or delay several forms of selenium deficiency syndromes (Mahan et al.,1991; Mahan et al., 2000).

Antioxidant status of an animal may affect the ability of phagocytes to migrate to the site of infection or inflammation. Marcsh et al. (1986) explained a process by which immune function might be compromised by both vitamin E and Selenium deficiency, thus reducing thymic growth. Lymphoid organs are the major targets of deficiency of these two nutrients. Free radicals are destroyed in blood by an antioxidant, thereby maintaining the structural and functional integrity of cells (Chew, 1995). Therefore, antioxidants are necessary for a healthy immune system, which plays an important role in growth and performance of animals under normal or stress conditions.

Recent studies on Vitamin E and selenium have focused on antibody production, disease resistance, while fewer studies have focused on cytokine production and growth (Finch and Turner, 1996). The stress following an immune system challenge is characterized by increased production of proinflammatory cytokines such as interleukin (IL-1, IL-6-8) and tumor necrosis factor α (TNF- α), produced by monocytes and lymphocytes. Therefore, high levels of cytokines reduce feed intake and redistribute nutrients away from growth towards the immune system. Liposaccharide (LPS) immune challenge is a model used to mimic a case of infection and study the immune response (Klasing et al., 1991; Rakhshandeh et al., 2010; Kim et al., 2012). Klasing (1988) reported that high levels of cytokines in the blood increase body temperature, resting energy expenditure, decrease feed intake, and alters nutrient metabolism. Research into

antioxidants such as vitamin E and selenium is ongoing to determine their ability to prevent stress-related molecule production induced by LPS, such as peroxides and cytokines (Cadenas and Cadenas, 2002).

CHAPTER II

REVIEW OF LITERATURE

Source and metabolism of vitamin E

Discovered in 1922 by Evans and Bishop, laboratory research in rats demonstrated that deficiency of vitamin E caused the female to abort and the male to become sterile with testicular degeneration. Vitamin E deficiency symptoms in swine were not described until more than 25 years later (Adamstone et al., 1949). Vitamin E has eight structurally similar, naturally-occurring compounds that have similar function. Vitamin E is a generic term including tocopherol and tocotrienol. Alpha-tocopherol is the most active form. Dl-alpha-tocopherol acetate is the most commonly used form in a swine diet because it has a longer shelf life and greater stability.

Alpha-tocopherol is a fat soluble vitamin essential for all species. Therefore, there are several reports regarding its supplementation to livestock. Recent studies on vitamin E have supported its importance in protecting against free-radical damage, reinforcing the immune system, and preventing heart disease and other disease cases (McDowell, 2000). There are eight naturally occurring forms of Vitamin E: β and δ tocopherols and α,β, γ, and δ tocotrienols (Evans et al., 1936 ; Whittle et al., 1966). The naturally occurring

form of Vitamin E is α-tocopherol acetate, which is degraded in the small intestine, but the acetate ester remains intact.

Sitrin et al. (1987) reported that Vitamin E absorption is related to fat digestion and bile facilitates the absorption by the action of pancreatic lipase. The small intestine is the first site of absorption. Esters are hydrolyzed in the gut wall, and the free alcohol enters the intestinal lacteals and is transported via lymph to the general circulation. Polyunsaturated fatty acids (PUFAs) inhibit the absorption whereas medium-chain triglycerides enhance absorption.

Vitamin E in blood is attached mainly to lipoproteins in the globulin fraction within cells and occurs mainly in mitochondria and microsomes. The tocopherol is transported to the liver and released in combination with low-density lipoprotein (LDL) cholesterol. The rate of absorption of different tocopherols and tocotrienols is in the same order of magnitude as with their biological activity. The absorption of α-tocopherol is best, with γ-tocopherol absorption being 85% that of α-forms and having the most rapid excretion. Vitamin E activity found in plasma is in α-tocopherol forms. (Ullrey, 1998).

Placental and Mammary Transfer of Vitamin E

There is little placental and mammary transfer of vitamin E; however, most of it is found in colostrum (Van Saun et al., 1989). Mahan (1991) reported limited placental transport of α-tocopherol, making piglets susceptible to vitamin E deficiency. Blood vitamin E level is low at birth and increases after consumption of colostrum. Vitamin E content in lactating sows is low in older sows compared to gilts. Because there is not placental transfer, offspring rely only on colostrum and milk to meet their daily

requirement of vitamin E (Pinelli-Saavedraa and Scaifed, 2003). According to Mahan et al (2000), the concentrations of colostrum in vitamin E and Se decline with advancing age of the sow. Therefore, older producing sows are more likely to produce offspring with low vitamin E and selenium levels in blood (Mahan, 2000).

Biological Antioxidant function of Vitamin E in Swine

A number of investigators have reported the importance of Vitamin E including antioxidant attributes, enhancing the immune system, integrity and optimum function of the nervous, circulatory, muscular, and reproductive systems (Hoekstra, 1975; Sheffy and Schultz, 1979; Bendich, 1987; McDowell et al., 1996). Due to their synergism, it has been demonstrated that some functions of vitamin E can be fulfilled in part or entirely by traces of selenium or by certain synthetic antioxidants. Vitamin E function is also affected by the sulfur-containing amino acids methionine and cysteine.

Vitamin E plays an important role as an intercellular and intracellular antioxidant. Vitamin E is also part of the intracellular defense against the different reactive oxygen and free radicals that in high number can cause damage to the cell through oxidation of unsaturated phospholipids (Chow, 1979). Alpha-tocopherol in the cell works as a quenching agent; thus helping the removal of free radical molecules within the cell. (Gardner, 1989; Herdt and Stowe, 1991).

Free radicals can damage biological systems (Padh, 1991). Superoxide anion radical, hydrogen peroxide, hydroxyl radical, and singlet oxygen are reactive oxygen species, which are produced in the course of normal aerobic cellular metabolism. Moreover, oxygen radicals produced by phagocytic granulocytes undergo respiratory

bursts to destroy intracellular pathogens. However, excess production of these oxidative products causes damage to healthy cells. Antioxidants such as vitamin E and selenium serve to stabilize these free radicals, thereby maintaining the structural and functional integrity of cells (McDowell et al., 2000). Therefore, the importance of antioxidants is that they enhance the immune system and health of humans and animals by protecting the cell against oxidative damage and enhance the rigidity of cell membrane. Vitamin E and selenium have a close relationship in their antioxidant function (Chew, 1995).

Source and metabolism of Selenium

Several forms of selenium are found in the body as part of proteins. The most common form of the element that enters the body are as two amino acids including selenocysteine and selenomethionine, mainly found in animals and plants, respectively (Burk et al., 1999). The primary site of absorption is the duodenum, and a small amount can be absorbed in the jejunum and ileum. Selenium is absorbed along with vitamin A, C and E, and a reduced form of glutathione peroxidase, which is more easily absorbed. However, heavy metals such as mercury can decrease absorption of selenium (Groff et al., 1996). According to Mahan et al. (2000), animal response to selenium sources varies, but mainly when comparing organic vs inorganic sources. Inorganic Se, sodium selenite and organic sel-plex are currently the forms advised by FDA to be used in livestock diets in the United States. Grains, animal products, and a selenium-enriched yeast source contains selenium in one of several amino acid analogs, but the most common one found in the grain and yeast product is seleno-methionine. There is evidence suggesting that the pig does not effectively utilize inorganic selenium above 0.1 to 0.2 mg with higher dietary concentrations being excreted mostly in urine. In contrast, organic Se when fed at

higher dietary Se levels is retained effectively. Organic selenium sources appear to be more satisfactory for animals since this form of selenium is more effectively transferred through the placenta and mammary tissue of adult swine: the period when young pigs are most susceptible to selenium deficiency (Chung et al., 1992).

Several studies have reported selenium action in aqueous cell media (mitochondrial matrix cytosol). The action of the enzyme glutathione peroxidase (GSHpx) of which selenium is a cofactor help with the removal of hydrogen peroxide and hydroperoxides. Thus, preventing oxidation of unsaturated lipid content in cells. Therefore, selenium protects fats within the cell membrane against oxidative damage. The oxidation of vitamin E helps to prevent oxidation of other lipid materials to free radicals and peroxides, thus enhancing protection of cell membranes from oxidative damage (Drouchner, 1976; Chung et al., 1992).

Placental and Mammary Transfer of Selenium

There is sufficient evidence showing selenium transfer across the placental membrane to the developing fetus in pigs. Research by Mahan et al. (1977) showed that concentration of selenium in serum and tissue of newborn piglets is increased when supplementation of selenium in the diet of sows increased from 0.1 to 0.5 mg.

Vitamin E and Selenium on Disease Resistance

A number of studies (Mahan and Maxon,1980; Mahan et al., 2000; Shunyi et al., 2015) have demonstrated several numbers of biological role performed by antioxidants in enhancing diverse aspects of the immune system by protecting the cell membrane against lipid peroxidation and free radicals. The function of vitamin E as an antioxidant is that it

could enhance immunity by maintaining the functional and structural integrity of the cell membrane, therefore, enhancing the immune system. A compromised immune system will negatively affect animal health and result in reduced performance of the animal by increasing susceptibility to infection because selenium has been reported to enhance the phagocytic effect, thereby leading to increased animal morbidity and mortality.

Vitamin E and Selenium play an important role in protecting leukocytes and macrophages during phagocytosis, the mechanism by which animals immunologically engulf and kill invading bacteria. Both vitamin E and Selenium may help these cells to survive the toxic products that are produced in order to effectively kill ingested bacteria (Badwey and Karnovsky, 1980). Macrophages and neutrophils from vitamin E-deficient pigs have decreased phagocytic activity (Burkholder and Swecker, 1990). Since vitamin E acts as a tissue antioxidant and aids in quenching free radicals produced in the body, any infection or other stress factors may exacerbate depletion of the limited vitamin E stores from various tissues. With respect to immunocompetency, dietary requirements may be adequate for normal growth and production; however, higher levels have been reported to positively influence the immune system (Machlin et al., 1975).

Phagocytic respiratory bursts of corticoid synthesis are permanent producers of free radicals that challenge the antioxidant systems of the animal. α-Tocopherol has been implicated in stimulation of serum antibody synthesis (Tengerdy, 1980). The protective effects of α-tocopherol on the health of animal may be involved with its role in protecting the cell membrane against peroxidation (Golub and Gershwin, 1985). Fritsche and McGuire (1996) reported a decrease of vitamin E in blood and liver concentrations in rats after an inflammatory challenge. Moreover, α-tocopherol has an immuno-enhancing

effect by virtue of altering arachidonic acid metabolism and subsequent synthesis of prostaglandin, thromboxanes, and leukotrienes. Under stress conditions, increased levels of these compounds by endogenous synthesis or exogenous entry may adversely affect immune cell function (Hadden, 1987).

In sows, vitamin E restriction depressed lymphocytes and polymorphonuclear cells for immune function (Wuryastuti et al., 1993). Antioxidants, including α-tocopherol, and selenium are important in resistance to viral infection. Vitamin E deficiency allows a normally benign virus to cause disease (Beck et al., 1994). In mice, enhanced virulence of a virus resulted in a myocardial injury that was prevented with vitamin E adequacy. Prolonged selenium depletion does not impair resistance to viral infection in calves (Reffett et al., 1988) or nematode infection in lambs (Jelinek et al., 1988; McDonald et al., 1989).

Disease susceptibility is an early consequence of selenium deprivation. Marginal selenium depletion has lowered the resistance of chicks to the protozoan parasite Eimeria tenella (Colnago et al., 1994). Mahan et al. (2000) reported that cellular immunity appears to be enhanced by the combination of both nutrients. Larsen and Tollersud (1981) demonstrated that pig lymphocytes responded to both Se and vitamin E in a phytohaemagglutinin response. A study conducted by Wuryastuti et al. (1993) demonstrated that pregnant gilts at 90 ds post-reproduction and (or) within 3 ds postpartum had improved cellular immune responses when supplemented with vitamin E (60 IU/kg) and Se (0.3 mg). Moreover, in response to both nutrients, cellular immune responses were noted to be increased in colostrum.

The efficacy of both nutrients appears to enhance the immunogenic response of the sow. High dietary levels of approximately 60 IU vitamin E/kg and 0.3 mg/kg of Se both appear necessary to maximize the immunogenic capability and to maintain herd health in adult reproducing sows.

Vitamin E and selenium on tissue protection

There is synergism between vitamin E and Se within tissues. Selenium has a sparing effect on vitamin E and delays the occurrence of deficiency signs. Likewise, vitamin E and sulfur amino acids partially protect against or delay the occurrence of several forms of selenium deficiency. Tissue breakdown has been reported in species fed diets deficient in both vitamin E and selenium, mainly through peroxidation because of the role that α-tocopherol plays in the rigidity of cell membranes. Peroxides and hydroperoxides are highly destructive to tissue integrity and can lead to disease development. (Mcdowell, 2000). It appears that vitamin E in cellular and subcellular membranes is the first line of defense against peroxidation of vital phospholipids. Selenium, as part of the enzyme glutathione peroxidase, is the second line of defense that destroys these peroxides before they have an opportunity to cause damage to membranes. (Mahan and Moxon, 1980). Therefore, selenium and vitamin E, through different biochemical mechanisms, are able to prevent diseases (Smith et al., 1974). To some extent, vitamin E and Se are mutually replaceable, but there are lower limits below which substitution is ineffective. In diets severely deficient in Selenium, vitamin E does not prevent or cure exudative diathesis, whereas addition of as little as 0.05 mg Se completely prevents this disease (Scott, 1980).

Mavromatis et al. (1999) observed a synergism between vitamin E and selenium when an additional 30 mg of dietary vitamin E/kg and injection of 0.5 mg/kg of selenium intramuscularly 3 times, on ds 30, 60 and 90 during gestation period of sows. The low level of selenium pigs were supplemented with both Vitamin E (20 mg/kg) and selenium (0.45 mg/kg). The additional vitamin E increased the serum concentration of IgG in piglets. Hoekstra (1975) suggested that vitamin E and selenium have a synergism, which is related to the process of antioxidation, wherein tocopherols tended to prevent oxidative damage to polyunsaturated fatty acid (PUFA) in cell membranes. Selenium, as part of selenoenzyme glutathione peroxidase, catalyzed the destruction of lipid hydroperoxides. These functions explain how these two nutrients play separate but interrelated roles in the cellular defense system against oxidative damage.

Requirement of vitamin E and selenium

Dove and Evans (1990), Mahan (1991), Mcdowell et al. (1996) concluded that the minimum vitamin E requirement of normal growth performance of pigs is approximately 0.30mg of diet which is higher than the NRC requirement (16 mg/kg). Vitamin E requirements are difficult to determine because of the interrelationships with other dietary factors and the content of PUFA in diet can increase the need of α-Tocopherol. Vitamin E requirements increase when there are high levels of PUFA found in oils such as corn oil, soybean oil and sunflower seed oil (Scott et al., 1982).

A number of researchers reported that supplements of 100 IU of vitamin E per kilogram of diet and 0.1 mg/kg of selenium did not entirely prevent deficiency lesions in weanling pigs afflicted with dysentery and fed 3% cod liver oil. Since the PUFA content

of membranes can be altered by dietary fats, it is not surprising that the dietary requirement for vitamin E is closely related to the dietary concentration of PUFA. When the level of PUFA in diet is high, more vitamin E is needed in the diet to decrease oxidation of PUFA (Hayes et al., 1969). The amount of vitamin E needed to maintain adequate growth and reproduction would not necessarily be enough or may not be the same as the amount needed to ensure optimal immune function (Weiss, 1998).

In swine, the inclusion of high levels of α-tocopherol in the diet may improve the immune system (Ellis and Vorhies, 1976 ; Peplowski et al., 1980). Infection, stress, exercise, and tissue trauma all increase α-tocopherol need (Nockels, 1991). Nockels et al. (1996) observed that for most sampled tissues, stress did not affect α-tocopherol concentration, although other indicators confirmed a deficiency. Vitamin E and Selenium requirements are greatly dependent on the content in the diet of both nutrients. As noted earlier, they are mutually replaceable above certain limits. For example, α-tocopherol has a sparing effect on selenium and reduces its requirement mostly in two ways; first overall, vitamin E help to maintain body Selenium in an active form. Secondly, α-tocopherol aids in preventing destruction of membrane lipids within the cell membrane, by doing so, α-tocopherol inhibits the production of hydroperoxides, therefore, less amount of the enzyme (GSH) will be needed to destroy free radicals. Selenium is known to reduce vitamin E requirement in 3 ways; first, it is needed to maintain the integrity of the pancreas, allowing normal lipid materials digestion and thus normal α-tocopherol absorption. Secondly, by reducing the amount of α-tocopherol needed for proper function of lipid membranes via glutathione peroxidase. Thirdly, it aids in retention of vitamin E in the blood plasma in some way that has not been clearly explained. (Glienke and Ewan,

1974). Vitamin E is one of the least toxic of vitamins; toxicity has not been seen in pigs. Bonnete et al. (1990) fed growing pigs a diet containing 550 mg/kg level of vitamin E and did not observe toxic signs.

Deficiency of Vitamin E and Selenium in pigs

In swine, most deficiency symptoms of vitamin E deficiency have been associated with Selenium. Because of the synergism of these nutrients, it is not clear to associate the deficiency to one nutrient, and thus scientists usually refer to vitamin E and Se deficiency. Heart disease and sudden death are often linked to vitamin E-selenium deficiency in swine. A number of cases have not shown clinical symptoms before the death of the animal (Michel et al., 1969; Trapp et al., 1970). One of the most common pathological lesions includes massive hepatic necrosis (hepatosis dietetics), cardiac failure, hemoglobinuria muscles, edema, nephrosis, esophagogastric, ulceration, icterus, acute congestion, and hemorrhaging in various tissues (Trapp et al., 1970; Piper et al., 1975). Additionlly, Trapp et al. (1970) observed a yellowish discoloration of adipose tissue.

In sows, mastitis-metritis-agalactia (MMA) syndrome have been reported in swine herds and was linked to vitamin E-Selenium deficiency. Other deficiency signs include spraddled rear legs in newborn pigs, gastric ulcers, poor skin condition, and infertility. Most of these deficiency symptoms were believed initially to be unrelated to vitamin E-Selenium deficiency. However, after supplementation with dietary vitamin E or injections of selenium and vitamin E, a noticeable reduction in these conditions occurred (Trapp et al., 1970). Whitehair et al. (1983) provided evidence that the mastitis,

metritis and agalactia (MMA), syndrome may be ameliorated by supplementation of the gestation-lactation diet with vitamin E and selenium. Conversely, when Guzman et al. (1997) fed alow vitamin E-Selenium at a young age for long period of time to boars, reproductive efficiency by boars decreased.

Mulberry heart disease is one of the diseases caused by vitamin E deficiency, it is characterized by mulberry appearance within the heart from hemorrhagic lesions. Piglets fed a diet deficient in vitamin E and Selenium had a low tolerance to injections of iron dextrose to prevent anemia. Necropsy lesion caused by selenium deficiency are similar to those of vitamin E deficiency. The symptoms include hepatic necrosis, edema of spiral colon and dystrophy of the skeletal muscle, reduce milk production, and impaired immune function (Peplowski et al.1980; Simesen et al., 1982).

Vitamin E-Selenium deficiency symptoms were observed on many swine farms in the 1960's. The deficiency problem was occurring during the post-weaning period, mortalities were around 10%. In older sows, these deficiencies caused prolonged farrowing times, poor milk let-down, high incidences of mulberry heart disease, lower litter size, fetal deaths, and weak lethargic pigs at birth. Younger pigs had white muscle, mottled livers, ceroid pigment in fatty tissue, and the death of rapidly growing pigs. Vitamin E and/or selenium deficiency compromise the immune system function, decrease growth performance and can lead to sudden death, thus contributing significantly to economic losses of the pork industry (Mahan, 2000).

Glutathione peroxidase

A number of immunologically distinct peroxidases uses glutathione (GSH) as a reducing substrate, important in helping to low level of selenium the peroxidation process. The first peroxidase to be identified and studied in detail was cytosolic glutathione peroxidase. The most abundant glutathione peroxidase in an animal is glutathione peroxidase 1, which accounts mostly for selenium in blood and liver. Glutathione peroxidase activity is the most studied enzyme to determine selenium status in serum (Floche et al., 1973). Glutathione peroxidase activity is used to measure selenium status in swine. Stabel et al. (1993) observed a compromised immune response when piglets were deprived of selenium. The production of reactive oxygen species (ROS) is a normal physiological process, including aerobic metabolism, oxidative phosphorylation, and in active neutrophils and macrophages occurring during the immune response (Fridovich, 1995). However, oxidative damage can happen when antioxidant properties are low and/or when oxidative stress is increased, therefore, increasing production of free radicals (Ibrahim et al., 2000). To avoid tissue and cell damage caused by excess free radicals, glutathione peroxidase has been demonstrated to fulfill this function. Glutathione peroxidase is in the cell cytosol where it plays the role as antioxidant, directly by reducing hydrogen peroxides and phospholipase A2 cleaved lipid hydroperoxides (Rotruck et al., 1973).

Development of the Immune System of the nursery pig.

The post-weaning phase is critical for the health of the piglet. Their immune protection provided by antibodies from Sow decreases. Piglets have to rely on their own immune system. At this age, piglets face challenges such as adaptation to the new environment, transportation, handling and change of diet from colostrum to solid feed (Mahan, 2000). The early development of a strong immune system at a young age is very important for securing the health of the animal and enhancing future growth and performance. Any compromise to a piglet during this period has repeatedly shown to negatively influence subsequent performance. Under stress conditions, the immune systems of piglets can face compromise and this affects nutrient metabolism (Kelley, 1980).

Immunology is a science field that investigates different mechanisms, allowing animals to distinguish their own cells and foreign pathogens. The two different immune system includes the innate immune system and the adaptive immune system. The innate immune system is developed and ready at birth. It includes phagocytic macrophages and dendritic cells. The adaptive immune system develops during exposure to the pathogen. Protection arises through humoral and cellular immunity by producing antibodies and white blood cells (Klobasa et al., 1981)

Most aspects of the immune system of piglets are immature during the development of active cellular immunity. Most of the antigen-presenting cells are lower

and the ability of intestinal T cells to respond to mitogens is undeveloped in neonatal pigs (Gaskins, 1998).

Similarly, the number of cells secreting immunoglobulins in bone marrow and spleen were lower between the first and fourth WK of age (Klobasa et al., 1981). The components of the immune system of the piglet are present at birth but are functionally undeveloped and several WKs of life are necessary before the immune system becomes fully developed (Brianchi et al., 1999).Therefore, newborn piglets mostly rely on the innate immune system and the colostrum for protection (Owens, 1988). Also, biochemical changes of the gut epithelium occur during the weaning period of newborn piglets.

Bianchi et al. (1999) reported that the immunological changes happened and correlated with inflammation of the gut. The immune system of the newborn piglets is undeveloped at birth because of lack of exposure to antigens.

Physiological development at weaning

Makkink et al. (1994) reported that the weaning period is followed by physiological and architectural changes of the GI tract. Additionally, change in form of feed and physiological changes in structure and function of intestinal enzymes at weaning decrease feed intake, negatively affecting performance. The GI tract has several functions, including digestion and absorption of nutrients, uptake of immunoglobulins by pinocytosis, enzyme secretions, secretion of mucus, and acting as a barrier against external pathogens. Several studies (Pluske et al., 1997; Boudry et al., 2004; Campbell et al., 2013) observed marked changes in the GI tract during weaning, including crypt

hyperplasia and villous atrophy. These changes negatively affect the digestive and absorptive capacity of the small intestine, causing diarrhea. According to Boudry et al. (2004), the development of the intestine is a structured process that involves the formation of a firm epithelium. To have a smooth viscosity of luminal contents and remove toxins, epithelial cells of the intestine have to fulfill the role, such as maintenance of the luminal barrier from external bacteria, digestive and absorptive functions (Pacha, 2000).

Lipopolysaccharide Challenge in Swine

Lipopolysaccharide (LPS) is a constituent of the outer membranes of Gram-negative bacteria (*E.coli*) that stimulates innate immunity. Lipopolysaccharide challenge is a well-established method used in immune response studies of pigs (Wright et al., 2000; Steiphen et al., 2015). Lipopolysaccharide challenge induces disease symptoms such as diarrhea, vormiting, lethargy, increased body temperature, and reduced feed intake (Johnson & Borell,1994; Webel et al., 2000). Lipopolysaccharide is recognized by the immune system as a pathogen-associated molecular pattern (PAMP). The lipopolysaccharide molecule is composed of lipid A and O side chain core oligosaccharide (Freudenberg et al., 2008). Lipid A is the PAMP responsible for activation of Toll-like receptor 4 (TLR4), a receptor involved in the inflammatory response (Wyns et al., 2015). A number of receptors including CD14, TLR4 and MD-2 recognize the molecule. Macrophages, monocytes, lymphoid cell, and non-immune system cells express CD14, TLR4 and MD-2 (Brian et al., 2003; Bryant et al., 2010).

When lipopolysaccharide binds to immune cells, it initiates a signaling cascade that activates transcription factors (NFkB) to upregulate the gene expression of various proinflammatory cytokines (TNF-α, IL-1β , IL-6), cyclooxygenase (COX)-2, and induction of the eicosanoid pathway, including prostaglandins (PGs) and other molecules (Bryant et al., 2010). The main source of eicosanoids is arachidonic acid, moreover, there is upregulation and release of different kinds of protein and acute phase proteins (APP) in the liver. Pig major acute phase protein (pig-MAP), C-creative protein (CRP), and serum amyloidA (SAA) have reported being the major APP in pigs (Wyns et al., 2015).

According to Johnson et al. (1997), lipopolysaccharide has been reported to activate the hypothalamus-pituitary-adrenal (HPA) axis through stimulation of proinflammatory cytokines. The outcome is increased secretion of glucocorticoids, which negatively affects growth hormone secretion (Lu & Murphy, 1989). The most widely used LPS in swine is the stereotype 0111:B4 (William et al., 2009). High production of proinflammatory cytokines negatively affects growth and performance and impairs nutrient utilization as nutrients are directed toward the immune system instead of growth (Johnson, 1997).

Cytokines

Cytokines are pro-inflammatory peptides involved in innate and adaptive immune response (Jonson, 1997). Cytokines are produced by white blood cells, such as lymphocytes and macrophages, but they can also be produced by fibroblasts, epithelial and endothelial cells (Pie et al., 2004). Cytokines such as TNF-α, IL-1β and IL-6β can act on the brain and other target organs to alter metabolism and inhibit growth (Johnson, 1997). High levels of cytokines in the brain have been reported to reduce appetite

because of anorexia, increases muscle protein utilization, and increase degradation of lipids in adipose tissue (Pie et al., 2004). These peptides play an important role in the regulation of the immune response, including systemic and local inflammation, metabolism, cellular proliferation, and repair of tissue (Arango Duque & Descoteaux, 2014).

Some cytokines can have a similar mode of action and may act on different cell types (Huynh et al, 2007). Therefore, these molecules are classified as anti-inflammatory and pro-inflammatory cytokines (Arango Duque & Descoteaux, 2014). Johnson (1997) noted an increase in proinflammatory cytokines after an immune system challenge. Immune cells such as macrophages and monocytes synthesize TNF-α mostly as a 26-Kd protein. This protein undergoes enzymatic processing by a metalloproteinase to the biological active form 17-Kd. There are two receptors (Type I and II) of TNF-α, since TNF-α is capable of stimulating the production of other inflammatory molecules such as IL-1 and IL-6, tumor growth factor, and arachidonic acid, which is used to produce prostaglandin E2 and prostacyclin. Thus, the immune reaction is initiated by TNF-α, and a cascade of responses contribute to the recruitment and activation of inflammatory cells (Marikovsky et al., 2003).

Interleukin-1β is synthesized as an inactive 31-kD precursor protein. The biological activity of IL-1β is mediated by the type I receptors. Injection of recombinant IL-1 can induce anorexia and other disease symptoms. Macrophages and monocytes are not the only source of IL-1. They can also be produced by B cells, NK cells, dendritic cells, epithelial cells, and fibroblasts. There are 3 forms of IL-1, such as IL-1α, IL-1β and IL-1Ra. TNF- α and IL-1β are involved in endogenous pyrogen release after an immune

challenge in response to stress, infection, and lesions. During inflammation, the production of APP from the liver and its mode of action on the central nervous system to induce sickness and prostaglandin production stimulated by IL-1β (Johnson 1997; Arango-Duque & Descoteaux, 2014). Moreover, IL-1 increases the expression of histamine and initiates vasodilation and specific inflammation. All 3 forms of IL-1 compete for the same receptors (Arango-Duque & Descoteaux, 2014). High levels of pro-inflammatory cytokines have been reported to decrease feed intake, increase energy expenditure, increase body temperature, and cause fever. These cytokines can induce adipose tissue cells to secrete leptin, a hormone that acts on the central nervous system to decrease feed intake and increase energy expenditure (Klasing, 1988; Johnson, 1997).

Summary

At weaning, nursery pigs are subject to many stressors, including, change in environment, form of diet and handling; therefore, oxidative stress of these animals is increased. Piglets have to overcome these stressors to improve growth performance. Although at this age the immune system of piglets is undeveloped, newborn pigs rely on the immune protection provided in colostrum by the sow. Nutritionally, antioxidants such as vitamin E and selenium have been reported to decrease free radicals thereby improving growth performance and immune response.

Most of the research in this area have focused on the effect of these antioxidants on meat quality, reproduction, antibody production, and performance, however, little work has been done on the immune response of nursery pigs following an LPS challenge. Antioxidants can help decrease oxidative stress and proinflammatory cytokine produced

during an acute immune challenge. Since selenium is toxic at high levels the allowable amount of selenium recommend by the FDA is 0.3 mg/kg of diet. However, vitamin E is not toxic, therefore, high levels of vitamin E can be fed to improve growth performance and immune response. Furthermore, more studies are needed to understand the positive effect of antioxidants on immune response following an immune challenge.

CHAPTER III

Effect of dietary vitamin E and selenium on growth performance and immune response of nursery pigs following an immune challenge

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Abstract: Nursery pigs undergo stress during weaning periods. At this young age the immune of pig has not fully developed. Antioxidants such as vitamin E and selenium have been shown to protect the cell from oxidative damage. This protection from oxidative could enhance the immune system. Antioxidant might reduce the lipopolysaccharide (LPS) induced expression of proinflammatory cytokines. As LPS challenges induce sickness behavior and stress in pigs, we hypothesized that vitamin E and selenium supplementation would reduce proinflammatory cytokines production such as tumor necrosis factor α (TNF-α) and Interleukin 1 (IL-1), following an immune challenge. This reduction in cytokines would also improve growth performance. Two hundred eighty mixed sex piglets (PIC 380) with an initial BW of 5.8 kg were used in a 36 d study. Pigs were housed 10 pigs/pen (5 gilts and 5 boars), piglets were blocked and stratified based on sex and BW. Pigs were randomly allotted to 4 dietary treatments (0.15 mg/kf od Se and 16, .03, 32 vitamin E, and both Sel 0.3 mg/kg and vitamin E and). On d 21 and 22, 4 pigs from each pen were challenged with LPS, *E.coli* O111:B4 suspended in

a 9 g/L of sterile saline solution for a final dosage of 25 µg of LPS/kg of BW (Bible, 2013; Mandali et al., 2000). Pigs were weighed once per WK to determine ADG, ADFI and G:F. Serum samples used to analyze IL-1 and TNF-α concentrations using ELISA Kit, and plasma samples were used to determine the enzyme glutathione peroxidase activity. Data were analyzed using the PROC GLM procedure in SAS. The experimental unit was pen. Vitamin E alone did not improve ADG, ADFI and G:F, however, there a significant different on ADG and G:F in pigs fed 0.3 mg/kg of Se between d 21-36 of the study. There was a numerical increase in BW of pigs fed 32 IU vitamin E and 0.3 Se. Pigs fed 0.3 mg/kg Se and vitamin E significantly (P=0.04) improved overall G:F between (d 8-36) compared to pigs fed 0.15 mg/kg of selenium. Proinflammatory cytokines tumor necrosis factor α (TNF-α) and Interleukin IL-1 were increased at 3 h following LPS challenge. Dietary treatment did not decrease TNF-α) and IL-1 concentration in serum nor the increased temperature following the LPS challenge. supplementation of 0.3 mg/kg of Se improved ADG and G:F of weaned pigs between d 21-36 of the experiment. Overall, pigs fed 0.3 mg/kg of Se and vitamin E 32 IU/kg improved G:F between d 8-36 compared to pigs fed 0.15 mg of Se and vitamin E 16 IU.

Introduction

Overcoming stressors such as transportation and environmental changes are some of the challenges that nursery pigs have to undergo during weaning. Piglets have a limited ability to mount an immune response when weaned at a young age. Stressors can decrease feed intake and expose the pigs to disease infection thereby negatively affecting growth performance (Mahan, 2000). Reactive oxygen species (ROS) are generated by

stress and or LPS challenge (Dikalov, 2002). Weaned pigs need a functional immune system to combat the oxidative stress that occurs after weaning (Kick et al., 2012).

Oxidation of lipid membranes, intracellular proteins and DNA occur when ROS overcome the antioxidant defenses of pigs (Bartlett and Stadman, 1997), and can upregulate gene expression of inflammatory cytokines (Manning, 1997). High levels of cytokines in the brain have been reported to reduce appetite, increase protein muscle degradation and increase degradation of lipids in adipose tissue (Pie et al., 2004). In addition, there is impairment of nutrient metabolism as nutrients are directed toward immune cells rather than growth (Johnson, 1997). To minimize the oxidative stress occurring during weaning period, dietary vitamins and minerals have been examined (Mahan et al., 1991; Boudry et al., 2004). Antioxidants such as vitamin E and Se have been investigated for their role in destroying free radicals thereby improving cell integrity (Bartlett and Stadman, 1997). Several experiments have reported the importance of vitamin E including; antioxidant attributes, enhancing the immune system, integrity of the cell and reproductive benefits (Bendich, 1987; Hoestra, 1975; Mahan and Maxon ,1980; Sheffy and Schultz, 1979).

The amount of vitamin E required to improve the immune response is still undefined. Pinelli-Saavedra (2003) reviewed several studies related to vitamin E on growth performance, reproduction and immune function suggested that to improve the immune system, it is necessary to increase the dietary vitamin E 2 to 10 times above the NRC recommendation. Additionally, reports on beneficiary effects on vitamin E and Se depend on the sources of these two nutrients, organic vs inorganic, dietary vs injectable. Jonathan et al (2003) noted a decrease in proinflammatory cytokines such as tumor

necrosis factor α (TNF-α) and Interleukin IL-1 in mice when α-tocopherol was added to the diet.

Because the antioxidant function of vitamin E is closely related to Se, and Se has been shown to act in aqueous cell media (cytosol and mitochondrial matrix) by destroying hydrogen peroxidase and hydroperoxides via the enzyme glutathione peroxidase (GSHpx) of which it is a cofactor. We hypothesized that supplementation of Vitamin E and Se would improve growth performance and immune response of weaned pigs following an acute LPS challenge.

Objectives of the study

The objectives of this experiment were to determine; 1) the main effect of increased Se, 2) the main effect of increased vitamin E, and 3) the interactive effects of increased Se and vitamin E on the growth performance and immune response of nursery pigs. The parameters studied included; ADG, ADFI, G:F ratio, glutathione peroxidase activity and cytokine production (TNF-α, IL-1).

Materials and methods

Experimental design, Animals, Housing, and Diets

This experiment was conducted at the swine research unit at Oklahoma State University (Stillwater, OK) and all procedures were approved by the Animal Care and Use Committee of Oklahoma State University.

Two hundred eighty mixed sex piglets (PIC 280) with an initial BW of 5.8 kg were used in a 36 d study. Pigs were housed in pens containing 10 pigs/pen (5 gilts and 5 barrows). Pigs were weaned at 3 WK of age.

Piglets were blocked and stratified based on sex and BW. Pigs were housed in the nursery facility with control of environmental temperature and ventilation. The first WK the temperature was set at 31.1^0 C and it decreased by 1 degree C every WK. Pigs were fed a common corn-soybean meal based diet (0.15 mg of Se and 16 mg of Vitamin E) during the first WK of the experiment. At d 8 of the experiment, feeders were cleaned and weighed before feeding the experimental diet, which was in the second phase of the nursery (N2). Pigs were randomly allotted to 4 dietary treatments including: 0.15 mg/kg Se and 16 IU vitamin E, 0.3 mg/kg of Se and 16 IU of vitamin E, 0.15 mg/kg, 32 IU/kg vitamin E and both vitamin E 32 IU/kg and Selenium 0.3 mg/kg with 7 replicates per treatment. Vitamin E premix was obtained by mixing 0.45 kg of vitamin E 50 powder (α-tocopherol acetate) and 4.5 kg of corn. The experimental diets were fed from d 8 to 36 of the experiment. Both feed and water were provided ad libitum.

To meet the nutritional requirements of NRC (2012), the experimental diets were formulated in two different phases: N2 and N3 (Table 3.1). N2 diet was fed from d 8 to 21, the N3 diet was fed from d 21 to 36. Both diets were formulated as basal diets, and both ingredients selenium (sel-plex) and vitamin E (E 50) were added slowly during the mixing process. After weighing the basal diet, it was poured in the mixer for 2 minutes before adding 0.15 mg/kg of Se and 16 IU of vitamin E, 8 minutes later feed was collected from the mixer and stored in a 22 kg bags.

The feed was recorded for each pen before feeding, and feed intake was calculated weekly after weighing feeders. The feed was provided in a multiple-hole stainless steel feeder and water was provided by a water nipple. Pigs were weighed once a WK to determine ADG, ADFI and G:F.

Diet Analysis

All analyses were performed at Servitech (Dodge City, KS). Diets were analyzed for moisture, DM, CP, CF, crude fat, ash, digestible energy DE, ME, NE), calcium, phosphorus, magnesium, potassium, sulfur, sodium, iron, zinc, manganese, copper, and selenium.

Data Collection

Blood samples were drawn from the anterior vena cava (jugular) in the supine position using a 20 gauge 3.8 cm vacutainer needle with a 10 mL sterile serum tubes with anticoagulant, and a 3 mL sterile plasma tube (BD, Franklin Lakes, NJ). Blood samples were placed on ice after collection and stored at 3-5^{o}C overnight, then centrifuged for 10 minutes at 2,000 x *g* speed to separate the serum or plasma. Afterward, the serum and plasma were collected using a plastic disposable transfer pipet and placed into microcentrifuge tubes and stored at -20^{o}C for further analysis.

Escherichia coli Lipopolysaccharide Challenge

Lipopolysaccharide (LPS) is a constituent of the outer membranes of gram-negative bacteria (*E. coli*) that stimulates innate immunity. LPS challenge is considered a well-established method that is safe and has been used in immune response studies (Steiphen et al., 2015). Prior to LPS challenge, 4 pigs (2 males and 2 females) from each pen were randomly selected and ear tagged. On d 21 and 22, the selected pigs were

subjected to LPS challenge. For the purpose of analyzing immune response, *Escherichia coli* LPS O111:B4 (Sigma-Aldrich Co.; St. Louis, MO) was suspended in a 9 g/L of sterile saline solution for a final dosage of 25 µg of LPS/kg of BW (Bible, 2013; Mandali et al., 2000). The suspension was kept in a cold storage prior to injection.

Plasma and serum were collected at h 0, 3, and 6 post-injection using tubes containing heparin for plasma and red tubes for serum. Rectal temperature and BW were recorded prior to sample collection. Samples were kept in an ice chest after collection, then sample were centrifuged for 10 minutes at 2,000 x g to separate the serum or plasma according to the instruction of the manufacture and kept in the freezer (-20^0 C) for lab analyses. The time for sampling after LPS injection was chosen based on the maximal peak obtained for concentrations of (TNF-α) and (IL-1β) (Mandali et al., 2000; Warren et al., 1997; Webel et al., 1997). Hour 0 was used to calculate the changes in rectal temperature and percentage of BW. Serum samples were used to analyze IL-1 and TNF-α concentration using ELISA Kit from R and D system.(Mandali et al., 2000)

Blood Analysis

Serum samples from h 0 and 3 were analyzed in duplicate for TNF-α, IL-1β and plasma was used for glutathione peroxidase activity. An enzyme-linked immunosorbent assay (ELISA) kit was used to measure the concentration of TNF-α and IL-1β (R&D Systems, Inc., Minneapolis, MN). Samples were analyzed according to the instructions of the manufacturer.

The Quantikine porcine TNF-α immunoassay is a 4.5-h solid phase ELISA designed to measure TNF-α in serum or plasma samples, it contains *E-coli*-expressed

recombinant porcine TNF-α and antibodies raised against the recombinant factor. The ELISA test uses the quantitative sandwich enzyme immunoassay method. Similar method was used for IL-1β quantification.

The control, standard, and samples were added to the wells with a pre-coated monoclonal antibody specific for each porcine cytokine. The standard was reconstituted with calibrator diluent RD6-33 before putting it on the plate with a pippet. After incubation for 2 hs, the unbound substances were washed away, and an enzyme-linked monoclonal antibody specific for the measured cytokine was added to the wells to bind the cytokine immobilized during the first incubation. Two h later after the second incubation, the plate was washed to remove any unbound antibody-enzyme reagent. Later, the substrate solution was added to the wells. The color developed in proportion to the number of cytokines bound in the initial step. The developed color was blue, the stop solution was added, and the color changed to yellow. The intensity of the color was measured at 450 nm with the correction wavelength set at 570 nm. The sample values were then read off the standard curve. In the TNF-α ELISA assay, the h 3 samples were diluted 10-fold using the RD6-33 regent from the ELISA kit.

Statistical Analysis

Data were analyzed using the PROC GLM in SAS (version 9; SAS Institute, Inc., Cary, NC) appropriate for a factorial arrangement of treatments in a randomized complete block design. The statistical model included the effects of treatment, block, and their interaction. The main effect of vitamin E, Selenium, and their interaction was tested using orthogonal contrasts. Pen served as the experimental unit. Effects were considered significant at $P \leq 0.05$ and P-values < 0.1 were considered tendencies**.**

Results

Growth performance

The growth performance measures are presented in (Table 3.2). No differences (*P* = 0.13) were observed among treatments for BW. The initial BW was not different (P= 0.62), with an average BW of 6.9 kg. There were no differences among treatment between d 8-21 for ADG (*P*=0.80), ADFI (*P*=0.45) and G:F ratio (*P*=0.53). Also, no interaction (*P*=0.53) was observed for vitamin E and selenium. Between d 21 - 36, there was an improvement (*P* = 0.02) in ADG and G:F (*P*= 0.005) for pigs fed 0.30 mg/kg of Se compared to pigs fed 0.15 mg/kg of selenium, however, there was no difference (*P*=0.72) in ADFI. Overall, there was no statistical difference among pigs fed additional vitamin E or Se on ADG and ADFI (*P* = 0.15). However, pigs fed 0.30 mg/kg of selenium had improved G:F (*P* = 0.04) compared to pigs fed 0.15 mg/kg of selenium

Body weight and rectal temperature after LPS challenge.

There was a decrease in BW as expected following the immune challenge, however, no differences (P=0.14) in BW change were noted among treatments (Figure 3.1)

Between h 0 and 3 there was an increase in rectal temperature (Table 3.3), however no statistical differences (P= 0.58) were observed among treatments. Similar results were observed between h 3 and 6 (Figure 3.2)

In all treatment groups, the rectal temperature increased following LPS challenge, with the highest temperature observed at h 3 post-injection There was no significant differences (P =0.16) among treatments on rectal temperature following immune challenge.

Serum TNF-α and IL-1β analysis

There was no difference (*P* =0.15) among treatments for both h 0 and h 3 for serum concentration of TNF-α and IL-1β (Table 3.4). There was an increase in TNF-α and IL-1β at h 3 compared to h 0. Although there was a numeric decrease in TNF-α and IL-1β levels in pigs supplemented with vitamin E and selenium, no difference (P = 0.10) was observed. Dietary treatment did not affect the TNF-α and IL-1β production 3 h post-injection of LPS challenge.

Change in TNF-α and IL-1β following the immune challenge

Between h 0 and 3 following the LPS challenge, there was a numeric decrease in serum TNF-α and IL-1β (Figure 3.3 and Figure 3.4) for pigs fed increased Se and vitamin E. However, there was no statistical difference (*P*= 0.73) observed.

Discussion

Dietary supplementation of vitamin E and Se have been reported to have a positive effect on growth and performance (Mahan et al., 2000). However, the variation in performance of pigs supplemented with these nutrients depends on several factors such as duration of supplementation, route (injection or dietary), feed consumption, the composition of the diet, and stress (Pinelli-Saavedra, 2003).

Our findings demonstrate that the supplementation of vitamin E at 32 IU/kg of diet and Se at 0.15 mg/kg did not improve ADG, ADFI, G: F ratio, nor BW of the nursery pigs between d 8-21 of the current experiment.

These results agree with Upadhaya et al. (2015). In this experiment, vitamin E was fed at 300 IU/kg of diet in growing pigs and no improvement for ADG nor ADFI were observed. However, when n-3 fatty acid was combined with vitamin E, they noticed an improvement in ADG and a decrease in proinflammatory cytokine production by LPS challenged pigs.

Between d 21 and 36 of the current experiment, there was a Se effect. Pigs supplemented with 0.3 mg/kg Se had greater ADG and G: F compared to pigs fed 0.15 mg/kg of Se. This improvement might be attributed to Se utilization during protein turnover for the synthesis of sulfur-containing amino acids. Se can partially replace sulfur and help in methionine synthesis (Dean et al., 1957).

Between d 8-36, there was no statistical difference among treatments on BW, ADG, or ADFI. However, a significant improvement in G: F was observed for pigs supplemented with Se. The lack of difference among treatments compared to pigs fed low levels of Se on ADG and ADFI may suggest that treatment diet was not completely deficient in Se and vitamin E.

Following the immune challenge, between h 3 and 6, there was a numeric increase in pigs fed vitamin E in loosing less BW compared to Se fed pigs. It is well known that the acute immune challenge produces fever, anorexia, and a decrease in feed intake, which contributes to weight loss of challenged animals (Johnson et al., 1997; Frank et al., 2005).

Between h 0 and 3 there was an increase in rectal temperature change, however no statistical differences were observed among treatment in change. Similar results were

observed between h 3 and 6. Our experiment was the first to investigate the interactive effect of both vitamin E and Se of piglets following the immune challenge. Moreover, the rectal temperature was increased in all treatments, with a peak of high temperature observed at h 3 post-injection. Similar observations were reported in previous studies (Johson & Borell, 1994;Webel, et al. 1997; Wright et al., 2000; Frank et al., 2005; Bible et al., 2013).

LPS challenge was used to induce the acute phase to investigate the immune response. In commercial swine production, disease challenge, whether clinical or subclinical, can lead to the production of a number of immune system-related molecules such as acute phase proteins and cytokines (Wright et al., 2000). The acute phase response after an immune challenge with LPS includes increases in serum concentration of pro-inflammatory cytokines, stimulation of the hypothalamic-pituitary-adrenal axis, fever, reduced feed intake, and reduced growth (Johnson, 1997; Frank et al., 2005). Our results agree with White et al. (2000). Three h following immune challenge, there was an increase in proinflammatory cytokines such as TNF-α and IL-1β. Although in our experiment there was not a difference among treatments on TNF-α and IL-1β concentrations in serum, there were numeric decreases in TNF-α and IL-1β in pigs supplemented with both vitamin E and Se (Figure 3.3 and 3.4)

To improve immune response, it is necessary to increase (2-10) times the dietary amount of vitamin E above the NRC recommendation because Se can be toxic at high levels (Pinelli-Saavedra, 2003). In this experiment, *E.coli* LPS was chosen to mimic the immune system stimulation of nursery pigs raised under commercial conditions (Upadhaya et al., 2015). During the proinflammatory response, Prostaglandin E2 (PGE2)

production is also stimulated (Akaogi et al., 2006). Vitamin E has been reported to control (PGE2) synthesis in mice, therefore improving the immune system (Meydani, 1986). Kim et al. (2013) reported that Vitamin E blocks PGE2 synthesis by antagonizing peroxidation of arachidonic acid.

To test the hypothesis that vitamin E and Se would improve the immune system and additively attenuate the production of decreasing proinflammatory cytokines following an immune challenge of nursery pigs, we tried to mimic the acute challenge and production of proinflammatory cytokines. The high levels of TNF-α and IL-1 in serum may suggest that dietary treatment of vitamin E and selenium did not attenuate production of proinflammatory cytokines. A number of studies in vitro with cultured cells suggested that vitamin E (or another antioxidant) inhibit cytokine production from activated molecular cells. Mendez et al. (1995) reported that the LPS induced production of TNF-α by isolated macrophages when vitamin E or other antioxidant was infused in the cell culture. Our experiment disagrees with Webel et al. (1998) who observed a decrease in plasma proinflammatory cytokines (TNF-α, IL-1 and IL-6) in LPS challenged pigs when vitamin E was injected 3 d prior to LPS challenge. In our experiment, vitamin E was provided in the diet, and *E.coli*: O111:B4 was used with a final dosage of 25 μg/BW. In contrast, Webel et al. (1998) used 0.1 ml/BW of LPS (E.coli. serotype K-235). Vitamin E treatment had a numeric decrease in Il-1β at h 3 following LPS challenge. However, no statistical difference was observed between dietary treatments. There was a numeric decrease at h 3 in serum TNF-α change of pigs fed additional vitamin E and selenium. More studies are needed to understand the benefits of antioxidants such as vitamin E and selenium on the immune response of LPS challenged pigs.

Conclusion

The findings from the present experiment indicate that supplementation of 0.3 mg/kg of Se improved ADG and G:F of weaned pigs between d 21-36 of the experiment. Overall, pigs fed 0.3 mg/kg of Se improved G:F between d 8-36 compared to pigs fed 0.15 mg of Se and vitamin E 16 IU. Three h following the LPS challenge there was a numeric decrease in production of TNF-α and IL-1β in pigs fed additional Se and vitamin E. However, no significant difference was observed among treatments. Considering that selenium is toxic at levels above 0.3 mg/kg, it is preferable to supplement higher levels of vitamin E to pigs during periods of stress, as vitamin E has a lower risk of toxicity than selenium. More studies are needed to understand the precise amount of vitamin E needed to decrease proinflammatory cytokine production during an immune challenge.

Table 3.1. Composition of the basal diets druing Phase 1 (d0-8), Phase 2 (d 9-21), and Phase 3 (d 22-36)[a]

	Phase 1	Phase 2	Phase 3
Ingredients, %			
Corn, yellow dent	32.56	45.95	59.23
Soybean meal, 47.5% CP	15.00	22.00	34.30
Whey, dried	25.00	15.00	
Lactose	7.00		
Plasma spray-dried	6.00	3.00	
Blood cell spray-dried		1.25	
Fish meal, menhaden	6.00	3.00	
Soy protein concentrate	2.21	2.69	
Granulated fat	4.00	4.00	3.00
L-lysine HCl	0.17	0.24	0.25
DL-methionine	0.18	0.20	0.11
L-threonine	0.06	0.10	0.09
Dicalcium phosphate 18.5%	0.67	1.39	1.58
Limestone	0.44	0.49	0.74
Salt	0.50	0.50	0.50
Vitamin Premix	0.02	0.02	0.02
Mineral Premix	0.10	0.10	0.10
SelPlex	0.02	0.02	0.02
Choline Cl	0.03	0.03	0.03
Chemical composition of the diets			
Crude protein, %		22.0	24.7
Fat, %		5.2	5.7
Calcium, %		0.70	0.93
Phosphorus, %		0.65	0.80
Zinc, mg/kg		61	206
Copper, mg/kg		9	19
Selenium	0.15	0.15	0.15
Vitamin E, IU/kg	16	16	16

[a]Selenium and Vitamin E were added to the low level of selenium diet in the second and third phase to make the 3 dietary treatments.

Table 3.2 Growth performance of weanling pigs fed dietary selenium and vitamin E[ab]

						P <:		
	Low Se	Se	Vit E	Se/Vit E	SE	Se	Vit. E	Inter
BW d 8	6.8	6.8	6.8	6.9	0.04	0.62	0.62	0.62
BW d 21	11.1	11.2	11.2	11.2	0.41	0.86	0.76	0.84
BW d 36	18.5	18.9	18.8	19.1	0.52	0.13	0.27	0.79
D 8-21								
ADG, g	329	334	337	334	13	0.90	0.80	0.80
ADFI, g	409	424	417	419	11	0.45	0.91	0.53
G:F	0.80	0.78	0.80	0.79	0.03	0.53	0.71	0.82
D 21-36								
ADG, g	492	516	503	525	9	0.02	0.28	0.91
ADFI, g	742	740	750	742	14	0.78	0.72	0.88
G:F	0.66	0.69	0.67	0.70	0.02	0.005	0.40	0.99
D 8-36								
ADG, g	416	432	426	437	8	0.15	0.42	0.80
ADFI, g	587	593	595	593	9	0.88	0.70	0.66
G:F	0.70	0.72	0.71	0.73	0.01	0.04	0.42	0.94

[a]Least square means for 7 pens/treatment.
[b]Low level of selenium = 0.15 mg/kg Se and 16 IU/kg Vit E; Sel = 0.30 mg/kg Se and 16 IU/kg Vit E; Vit E = 0.15 mg/kg Se and 32 IU/kg Vit E; and Se/Vit E = 0.30 mg/kg Se and 32 IU/kg Vit E.

Table 3.3 Hourly BW and rectal temperature for pigs fed dietary selenium and vitamin E following an LPS challenge[abc]

						P≤ :		
	Low Se	Se	Vit E	Se/VitE	SE	Se	Vit E	Inter
BW, kg								
h 0	12.2	12.3	12.5	12.8	0.45	0.27	0.04	0.81
h 3	11.8	12.0	12.3	12.5	0.51	0.32	0.05	0.89
h 6	11.7	12.0	12.3	12.4	0.56	0.46	0.05	0.79
Temperature, ^{o}C								
h 0	39.7	39.5	39.5	39.5	0.12	0.24	0.31	0.32
h 3	41.0	40.9	40.7	41.0	0.18	0.37	0.60	0.16
h 6	40.3	40.3	40.2	40.3	0.18	0.68	0.72	0.90

[a]Least square means for 7 pens/treatment.
[b]Low level of selenium = 0.15 mg/kg Se and 16 IU/kg Vit E; Sel = 0.30 mg/kg Se and 16 IU/kg Vit E; Vit E = 0.15 mg/kg Se and 32 IU/kg Vit E; and Se/Vit E = 0.30 mg/kg Se and 32 IU/kg Vit E.
[c]LPS *E. coli* 0111:B4, 25 µg/kg of BW.

Table 3.4. Serum TNF-α and IL1- β concentrations of LPS challenged pigs at h 0 and 3 for pigs fed selenium and vitamin E

					P≤ :			
	Low Se	Se	Vit E	Se/Vit E	SE	Se	Vit E	Inter
TNF-α, pg/mL								
h 0	114	108	114	106	11	0.57	0.92	0.93
h 3	2,524	2,212	2,105	1935	298	0.42	0.25	0.81
IL-1β, pg/mL								
h 0	30	26	28	28	11	0.24	0.99	0.41
h 3	231	186	150	161	35	0.64	0.15	0.45

[a]Least square means for 7 pens/treatment.
[b]Low level of selenium = 0.15 mg/kg Se and 16 IU/kg Vit E; Sel = 0.30 mg/kg Se and 16 IU/kg Vit E; Vit E = 0.15 mg/kg Se and 32 IU/kg Vit E; and Se/Vit E = 0.30 mg/kg Se and 32 IU/kg Vit E.
[c]LPS Echerichia coli 0111:B4, 25 µg/kg of BW.

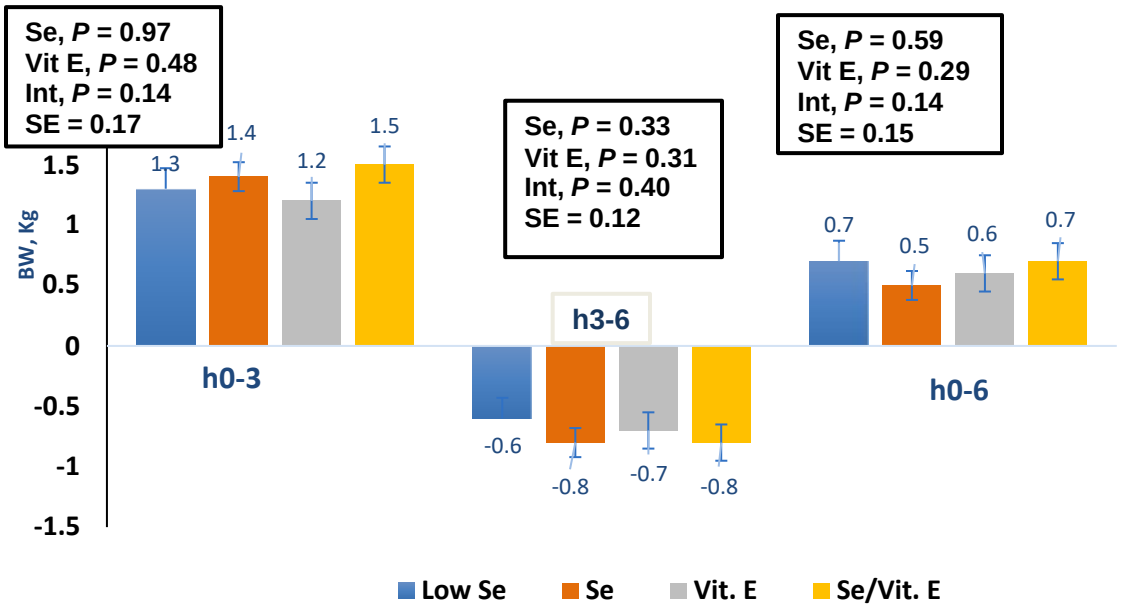

Figure 3.1. Effect of dietary Se and vitamin E on hourly BW change of nursery pigs following an acute lipopolysaccharide challenge (serotype of *E.coli* 0111:B4, 25 µg/kg of BW).

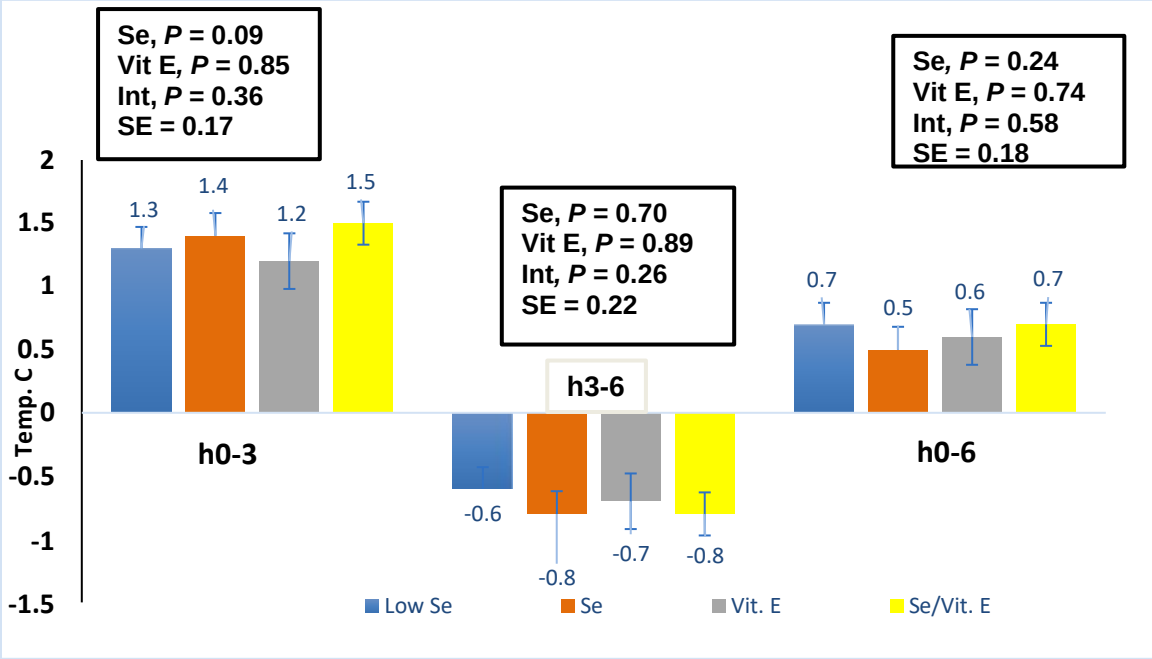

Figure 3.2. Effect of dietary of Se and vitamin E on rectal temperature change of nursery pigs following an acute liposaccharide challenge (serotype of *E.coli* 0111:B4, 25 µg/kg of BW).

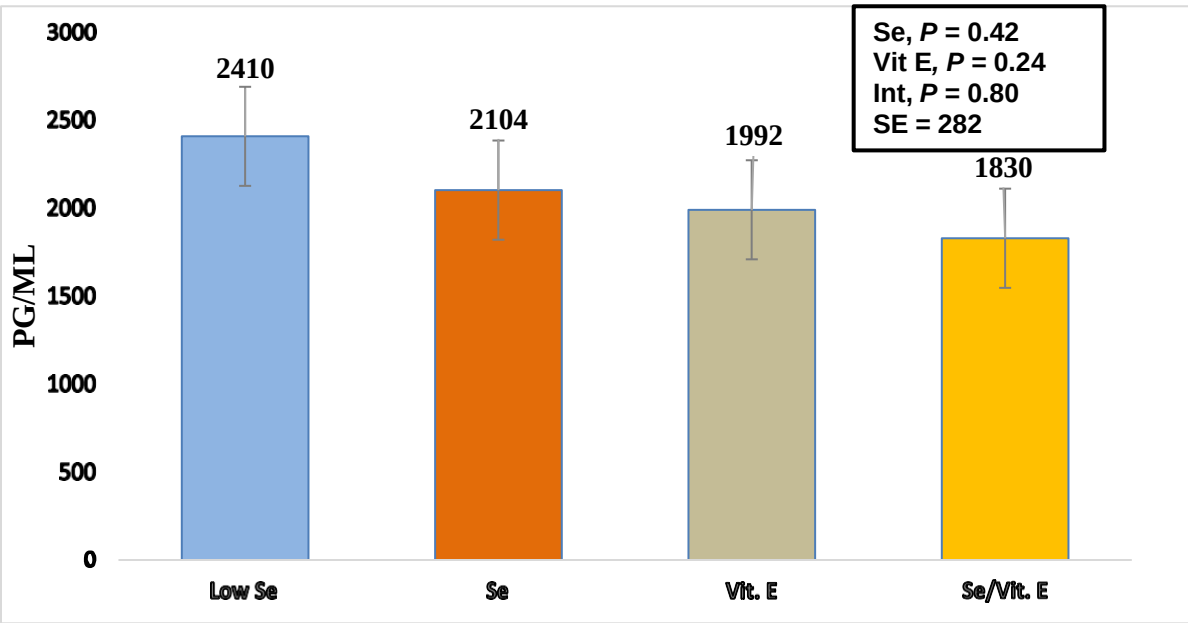

Figure 3.3. Effect of dietary Se and vitamin E on increase in serum TNF-α of nursery pigs following an acute liposaccharide challenge (serotype of *E.coli* 0111:B4, 25 µg/kg of BW).

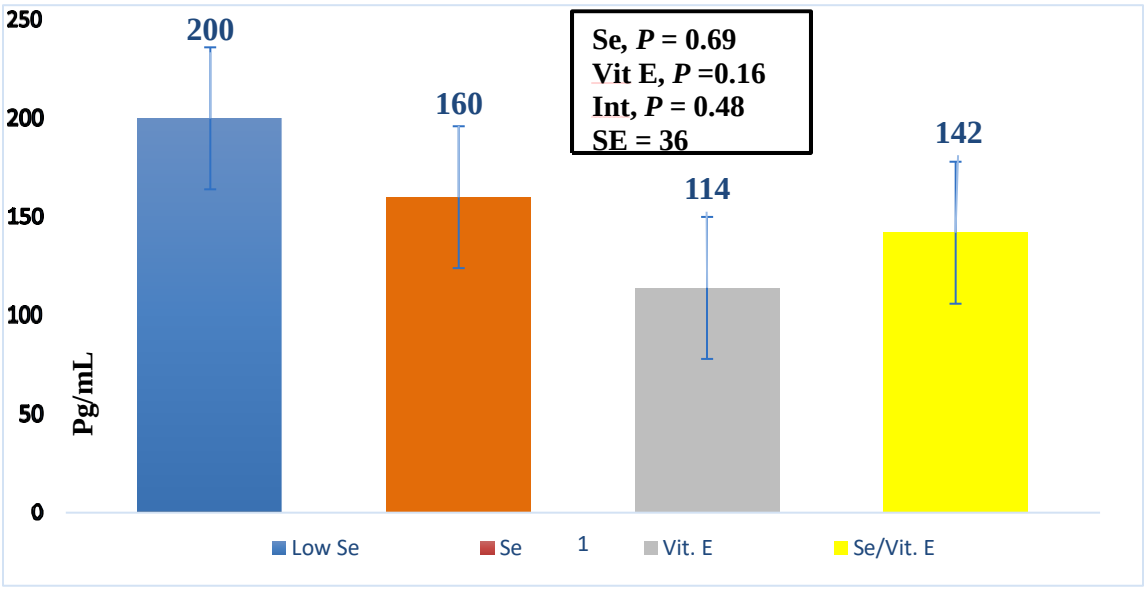

Figure 3.4. Effect of dietary Se and vitamin E on serum IL1β change of nursery pigs following an acute liposaccharide challenge (serotype of *E.coli* 0111:B4, 25 µg/kg of BW).

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APPENDICES

Table 1. Pen means for BW and average daily gain

				BW, KG			ADG, g		
Pen	Block	treatment	Pigs	d 8	d 21	d36	d8-21	d21-36	d8-36
10	1	D	10	7.2	11.1	18.8	29	512	413
16	1	B	10	7.1	10.9	18.5	292	503	405
17	1	C	10	7.1	12.0	19.3	372	488	434
24	1	A	10	7.1	12.1	20.1	379	537	463
5	2	B	10	7.1	11.6	19.4	355	512	441
13	2	D	10	7.0	11.6	19.7	358	539	445
22	2	C	10	7.1	11.4	19.0	333	503	424
25	2	A	10	7.1	10.7	17.6	278	458	375
14	3	C	10	6.9	12.3	19.3	417	512	468
15	3	A	10	7.0	11.4	19.0	337	512	431
23	3	D	10	7.0	12.4	20.9	421	567	499
27	3	B	10	7.0	10.9	19.0	306	553	438
3	4	C	10	6.9	11.4	19.5	341	546	450
19	4	B	10	6.9	11.5	19.4	355	521	444
26	4	D	10	6.9	11.3	19.1	333	524	436
28	4	A	10	6.9	11.4	18.7	347	482	420
4	5	B	10	6.9	11.1	18.9	372	482	468
12	5	A	10	6.9	11.1	18.3	330	479	410
18	5	C	10	6.9	10.9	18.3	302	485	400
20	5	D	10	6.9	10.7	18.7	295	521	416
1	6	C	10	6.6	10.4	17.8	292	497	402
2	6	B	10	6.6	10.4	18.5	288	542	424
6	6	A	10	6.6	10.8	17.7	323	458	395
8	6	D	10	6.7	10.8	18.7	313	528	428
7	7	D	10	6.3	10.5	17.8	323	485	410
9	7	C	10	6.5	10.4	17.7	299	491	402
11	7	A	10	6.4	10.9	18.2	309	519	421
21	7	B	10	6.4	11.3	18.7	376	494	439

Appendix Table 2. Pen means for average daily feed intake and gain to feed ratio

			ADFI, g			G: F		
Pen	Block	treatment	d 8-21	d 21-36	d8-36	d8-21	d21-36	d8-36
10	1	D	393	729	573	0.762	0.702	0.721
16	1	B	414	722	579	0.705	0.697	0.699
17	1	C	453	681	575	0.821	0.717	0.755
24	1	A	450	789	632	0.842	0.681	0.733
5	2	B	436	773	617	0.814	0.662	0.715
13	2	D	439	746	603	0.815	0.723	0.738
22	2	C	429	833	645	0.776	0.604	0.657
25	2	A	410	709	570	0.678	0.646	0.658
14	3	C	485	788	648	0.860	0.650	0.722
15	3	A	421	784	615	0.800	0.653	0.701
23	3	D	493	800	657	0.854	0.709	0.760
27	3	B	411	737	582	0.745	0.750	0.753
3	4	C	412	772	605	0.828	0.707	0.744
19	4	B	429	760	606	0.828	0.686	0.733
26	4	D	418	748	595	0.797	0.701	0.733
28	4	A	426	727	587	0.815	0.663	0.716
4	5	B	460	737	608	0.809	0.654	0.770
12	5	A	379	730	567	0.871	0.656	0.723
18	5	C	394	738	578	0.766	0.657	0.692
20	5	D	401	730	577	0.736	0.714	0.721
1	6	C	376	742	572	0.777	0.670	0.703
2	6	B	369	785	592	0.780	0.690	0.716
6	6	A	397	685	551	0.814	0.669	0.717
8	6	D	396	733	576	0.790	0.720	0.743
7	7	D	392	719	567	0.824	0.675	0.723
9	7	C	372	695	545	0.804	0.706	0.738
11	7	A	382	774	592	0.809	0.671	0.711
21	7	B	453	669	569	0.830	0.738	0.772

Appendix Table 4 Pen means of BW following LPS challenge

			BW kg			BW changes, g		
Pen	Block	treatment	hO	h3	h6	h0-3	h3-6	h0-6
10	1	D	11.9	11.4	11.3	-509	-113	-622
16	1	B	12.6	12.3	12.3	-260	-23	-283
17	1	C	13.4	13.3	13.4	-102	102	0
24	1	A	12.6	12.3	12.4	-396	158	-238
5	2	B	12.2	11.9	11.5	-328	-305	-633
13	2	D	13.2	13.0	12.8	-249	-158	-407
22	2	C	13.0	12.7	12.6	-328	-102	-430
25	2	A	12.7	12.5	12.3	-260	-158	-419
14	3	C	13.3	13.0	13.2	-328	215	-113
15	3	A	12.4	12.0	11.9	-373	-136	-509
23	3	D	14.7	14.3	14.4	-452	90	-362
27	3	B	13.4	13.1	13.1	-294	0	-294
3	4	C	12.5	12.3	12.2	-211	-90	-302
19	4	B	12.0	11.6	11.5	-392	-106	-498
26	4	D	13.1	13.2	13.3	121	151	271
28	4	A	11.8	11.3	11.4	-437	45	-392
4	5	B	11.9	11.4	11.5	-513	75	-437
12	5	A	12.6	11.9	12.0	-679	30	-649
18	5	C	12.3	11.7	11.7	-558	-15	-573
20	5	D	13.0	12.5	12.2	-558	-226	-784
1	6	C	12.1	11.9	12.1	-211	181	-30
2	6	B	12.4	12.2	12.3	-166	45	-121
6	6	A	11.4	11.1	11.1	-362	0	-362
8	6	D	12.5	12.1	12.2	-362	136	-226
7	7	D	11.9	11.4	11.3	-543	-121	-664
9	7	C	11.7	11.4	11.5	-271	45	-226
11	7	A	12.0	11.7	11.5	-317	-196	-513
21	7	B	12.4	12.1	12.2	-302	15	-287

Appendix Table 5 pen means for rectal temperature and changes in temperature following LPS challenge

			Rectal Temperature ^{O} C			Change in Rectal Temp		
Pen	Block	treatment	H0	h3	h6	h0-3	h0-6	h3-6
10	1	D	39.4	40.5	40.4	-1.1	-1	0.1
16	1	B	39.5	41.1	40.4	-1.6	-0.9	0.7
17	1	C	39.7	41	40.4	-1.3	-0.7	0.6
24	1	A	39.6	41.4	40.5	-1.8	-0.9	0.9
5	2	B	39.7	41.3	40.7	-1.6	-1	0.6
13	2	D	39.8	41.3	40.2	-1.5	-0.4	1.1
22	2	C	39.5	41.1	40.2	-1.6	-0.7	0.9
25	2	A	39.4	40.7	39.9	-1.3	-0.5	0.8
14	3	C	40	41	40.5	-1	-0.5	0.5
15	3	A	39.6	41.1	40.9	-1.5	-1.3	0.2
23	3	D	39.7	41.3	40.4	-1.6	-0.7	0.9
27	3	B	39.6	41	40.2	-1.4	-0.6	0.8
3	4	C	39.8	41.2	40.5	-1.4	-0.7	0.7
19	4	B	39.8	40.8	40.1	-1	-0.3	0.7
26	4	D	39.5	40.8	40.2	-1.3	-0.7	0.6
28	4	A	39.3	40.4	40.2	-1.1	-0.9	0.2
4	5	B	39.6	40.7	39.7	-1.1	-0.1	1
12	5	A	39.4	40.5	40.6	-1.1	-1.2	-0.1
18	5	C	39.9	40.9	40.6	-1	-0.7	0.3
20	5	D	39.5	41.5	40.6	-2	-1.1	0.9
1	6	C	39.6	40.9	40.1	-1.3	-0.5	0.8
2	6	B	39.2	41.1	40.3	-1.9	-1.1	0.8
6	6	A	39.5	40.8	40.1	-1.3	-0.6	0.7
8	6	D	39.9	40.8	40.5	-0.9	-0.6	0.3
7	7	D	39.8	41.2	40.4	-1.4	-0.6	0.8
9	7	C	39.6	41	40.2	-1.4	-0.6	0.8
11	7	A	39.5	40.7	40.2	-1.2	-0.7	0.5
21	7	B	39.8	40.6	40.4	-0.8	-0.6	0.2

2 males et 2 females subjected to IP LPS injection (0111:B4, 25 μg/kg BW)

Appendix Table 6. Pen means for TNF-α and changes in TNF-α Following LPS challenge

Pen	Block	treatment	h0	h3	hO-3	Fold
10	1	D	125.89	2926.94	2801.05	23.25
16	1	B	100.08	2617.78	2517.69	26.16
17	1	C	85.20	1387.08	1301.88	16.28
24	1	A	88.85	1880.45	1791.60	21.17
5	2	B	88.91	1894.11	1805.20	21.30
13	2	D	80.15	1513.01	1432.87	18.88
22	2	C	93.95	2594.43	2500.48	27.62
25	2	A	89.36	1304.49	1215.13	14.60
14	3	C	98.38	1414.91	1316.53	14.38
15	3	A	104.38	1960.92	1856.55	18.79
23	3	D	105.88	1448.94	1343.06	13.68
27	3	B	106.41	2501.51	2395.10	23.51
3	4	C	135.03	1954.15	1819.12	14.47
19	4	B	60.06	1589.85	1529.79	26.47
26	4	D	108.17	.	.	.
28	4	A	172.97	4525.69	4352.71	26.16
4	5	B	185.23	2000.97	1815.74	10.80
12	5	A	134.31	3759.59	3625.28	27.99
18	5	C	116.34	2331.26	2214.92	20.04
20	5	D	130.24	2828.17	2697.93	21.72
1	6	C	91.99	2672.64	2580.65	29.05
2	6	B	115.00	2554.24	2439.24	22.21
6	6	A	126.65	2365.90	2239.26	18.68
8	6	D	87.07	1387.07	1300.00	15.93
7	7	D	104.21	1510.52	1406.31	14.49
9	7	C	175.16	2387.30	2212.14	13.63
11	7	A	80.22	1876.52	1796.30	23.39
21	7	B	100.96	2330.29	2229.33	23.08

2 males et 2 females subjected to IP LPS injection (0111:B4, 25 µg/kg

Appendix Table 7. Pen means for IL-1β and changes in IL-1β Following LPS challenge

Pen	Block	treatment	h0	h3	hO-3	Fold
10	1	D	27.74	179.81	152.07	6.48
16	1	B	21.00	153.63	132.63	7.32
17	1	C	29.88	206.22	176.34	6.90
24	1	A	27.29	231.89	204.60	8.50
5	2	B	25.63	356.42	330.78	13.91
13	2	D	26.84	410.76	383.91	15.30
22	2	C	25.89	211.82	185.93	8.18
25	2	A	27.11	76.95	49.84	2.84
14	3	C	26.75	.	.	.
15	3	A	28.70	227.70	199.00	7.94
23	3	D	25.65	81.17	55.52	3.16
27	3	B	30.04	291.71	261.66	9.71
3	4	C	26.61	149.89	123.28	5.63
19	4	B	25.35	168.02	142.68	6.63
26	4	D	25.72	184.39	158.67	7.17
28	4	A	29.42	131.54	102.11	4.47
4	5	B	23.88	113.79	89.91	4.76
12	5	A	42.33	93.36	51.03	2.21
18	5	C	24.91	107.13	82.22	4.30
20	5	D	24.32	142.33	118.02	5.85
1	6	C	25.39	152.73	127.34	6.02
2	6	B	23.43	140.75	117.31	6.01
6	6	A	33.74	155.39	121.65	4.61
8	6	D	45.01	129.32	84.31	2.87
7	7	D	26.02	103.14	77.12	3.96
9	7	C	.	.	.	.
11	7	A	28.64	169.19	140.54	5.91
21	7	B	31.83	412.19	380.36	12.95

2 males et 2 females subjected to IP LPS injection (0111:B4, 25 µg/kg BW)

VITA

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Master of Science

Thesis: EFFECT OF DIETARY VITAMIN E AND SELENIUM ON GROWTH AND PERFORMANCE OF NURSERY PIGS NURSERY PIGS FOLLOWING AN ACUTE LIPOPOLYSACCHARIDE CHALLENGE.

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